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Separation of Hemi cellulose from Corn cobs by Alkali Pretreatment Method

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ABSTRACT:
Separation of lignocellulosic material into its individual components especially into cellulose and hemicellulose by various existing acid pretreatment methods are not promising as they result in low yield and high sugar loss along with the formation of inhibitors and moreover, high temperatures are required for separation. To over come these problems considerable effort has been made in the present work to rectify some of these drawbacks. In the alkali pretreatment (calcium hydroxide) method, the hemicellulose (which contains 70-90% xylose) concentration was found to be **32.83±0.13g/100g** of dry weight of corncobs **(86%)** and the concentration of inhibitors associated with hemicellulose i.e. phenolic, furfurals and lignin was 0.34±0.01g/100g, 0.0031±0.00040g/100g and 0.09±0.011 g/100g of dry weight of corncobs, respectively. However, in the present study with the existing acid pretreatment method, the concentration of hemicellulose obtained was **23.82±0.18g/100g** of dry weight of corncobs **(63%)** and the inhibitor compounds associated with hemicellulose i.e. phenolic, furfurals and lignin were found to be 3.8±0.2g/100g, 0.043±0.003g/100g and 6.0±0.25 g/100g of dry weight of corncobs, respectively. Further, we also found that the optimum temperature and time required for high yield of hemicellulose by alkali pretreatment method were **70^0C** and **5hrs,** respectively.

Keywords: Hemicellulose, corn cobs, alkali pretreatment, acid pre treatment.

INTRODUCTION

Corn cobs are renewable agricultural and corn based industries. It consists of lignocellulosic resources that are available in abundant quantities from the cellulose (42.0%), hemicellulose (38.5%), lignin (12.5%), Pectin (3.5%), Uronicacids (2.0%) and ash (1.5%)[1]. The percentage of these constituents can vary within a single plant, with age, stage of growth, and other conditions. Among the above constituents, cellulose and hemicellulose are very important from industrial point of view due to its wide range of application in the production of various value added products like ethanol and xylitol.

Cellulose is a polysaccharide of glucose units that serves as the main structural component of the corn cob's cell wall. Hemicellulose is a less complex polysaccharide that can more easily be broken down into simpler monosaccharides[2]. It is a short branched hetero polysaccharide with hexosons and pentosans. Lignin is a complex, non-carbohydrate (phenolic monomers) structural component which binds to cellulose and provides stiffness to the plant cell wall. Pectin is a complex carbohydrate of pectic acid and pectinic acid molecules, which is found in both the cell wall and between the cell wall of plants, helping to regulate the flow of water inbetween cells and keeping them rigid.

Pretreatment with concentrated acids such as H_2SO_4 and HCl which are used to treat the lignocellulosic materials, increase the release of fermentable sugars from cellulose. It also causes degradation of pentoses present in the hemi cellulose to form inhibitors like hydroxymethylfurfural,acetate, hydroxybenzaldehyde and vanillin [3]. Although acids are powerful agents for cellulose hydrolysis, concentrated acids are toxic, corrosive and hazardous and thus require reactors that are resistant to corrosion [4,5], which makes the pretreatment process very expensive. To make process viable dilute-acid hydrolysis has been successfully developed for pretreatment of lignocellulosic materials. Usually a sulfuric acid concentration below 2% has been of most interest, as it is inexpensive and effective. Further, dilute H_2SO_4 is mixed with biomass to hydrolyze cellulose and hemi cellulose to glucose, xylose and other sugars. However, the drawback of dilute-acid hydrolysis is formation of inhibitors and requirement of high temperature during pretreatment process.To overcome these problems, corn cobs are alkali pretreated which causes less sugar degradation, less inhibitors formation and also proved to be less expensive [6]. Therefore in the present study, an attempt has been made to extract hemicellulose from corncobs using 2%calcium Hydroxide.

MATERIALS AND METHODS

Raw materials

Different lignocellulosic materials such as hardwood, softwood, nut shells**,** corn cobs, cotton seed hairs etc. which are available locally at cheaper cost are screened for their hemicellulose content (Fig.1). Among these, corn cob was selected as suitable substrate for the experiments, as it has high percentage of hemicellulose (Fig.2).All the experiments were conducted in triplicate and the data was represented as mean value.

Hemi cellulose extraction by alkali pretreatment method

Hemicellulose extraction by alkali pretreatment method was carried out according to Rutenberg and

Herbst (1957) method [7]. Six grams (dry wt) of corn cobs was suspended in 60 ml of extracting solution (1:10 w/v) containing 2% of 1M $Ca(OH)_2$ and incubated for 5 h at 70 °C with occasional stirring. After cooling to 30-35°C, the mixture was centrifuged at 20°C for 20min at 18,000g. The supernatant was collected and the pH was adjusted to 4.5-5.0 with 6 N HCl with rapid stirring. Three volumes of cold ethanol (95%, 4°C) was slowly added to the supernatant with continuous stirring for 5 min and the precipitate formed was allowed to settle down for another 10-15 min. The bulk of the supernatant was separated which contains lignin fraction which can be used for other application. The precipitate (crude, rubber-like xylan pellet) was collected by low speed centrifugation at 20 °C for 10 min at 5000*g*.

Hemi cellulose extraction by Acid Pretreatment method

Hemi cellulose extraction was done according to Yang et al method *(2009*) [8]. 2.0% Sulfuric acid was used to pretreat oven-dried corncobs at a solid-to-liquid ratio of 1:10. (w/v) The mixture was autoclaved at 121°C/15 psi for 60 min. After autoclaving the liquid fraction was collected by filtration which was neutralized with 1M NaOH and detoxified with 2% activated charcoal (W/v) at 70^0C and the liquid fraction was analyzed for hemicellulose, lignin, phenolics and furfurals.

Analytical method

The quantification of hemicellulose was carried out by Rutenberg and Herbst method (1957) [7]. In this method the hemi cellulose pellet obtained during alkali extraction process was quantified by taking the dry weight of pellet, and the hemi cellulose obtained by acid pretreatment method was quantified using HPLC. The quantification of lignin was carried out by KenjiIiyam method (1990) [9] and absorbance was measured at 280nm using UV-VIS spectrophotometer (Systronics 117).

The quantification of phenolics was done according to Tanner and Brunner method (1987)[10] and absorbance was measured at 750 nm using UV-VIS Spectrophotometer (Systronics 117).

The quantification of furfurals was done according to Martinez et al (2000) method [11]. The test sample was suitably diluted and absorbance was measured spectrophotometrically at 280 nm using UV-VIS Spectrophotometer (Systronics 117).

Sugars in the hydrolyzate were estimated by High Performance Liquid Chromatography (HPLC) fitted with REZEX RPM COLUMN (8mm X 300mm) (phenomenex, USA). The samples were eluted with HPLC grade water at a flow rate of 0.6 ml/min at 75°C and detected with a differential refracto meter (RID).

RESULT

Composition of hemicellulose in different lignocellulosic materials

Among locally available lignocelluosic material (hardwood, softwood, nut shells, corn cobs, cotton seed hairs) corn cobs was found to have more percentage of hemi cellulose i.e. 38.5% (Fig.1)

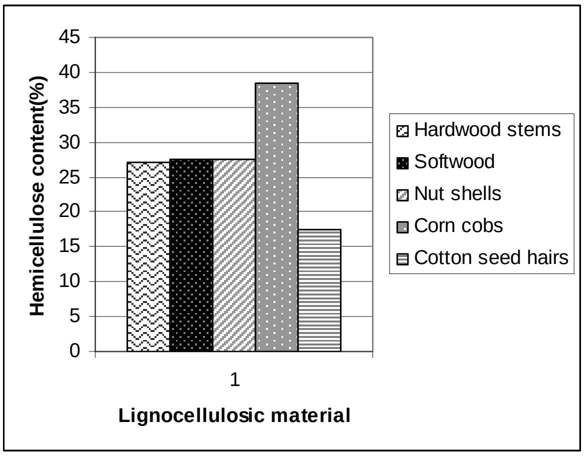

Fig.1 Composition of hemicellulose in different Lignocellulosic materials

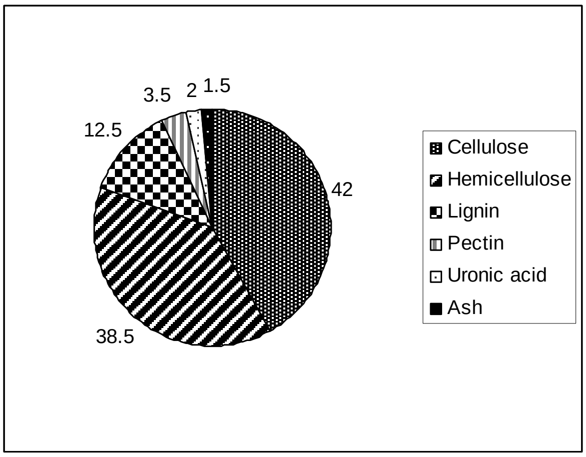

Fig.2 Composition of corn cob(%)

Corn cobs Composition

The composition of corn cobs was found to contain 42.0% of cellulose, 38.5% of hemi cellulose, 12.5% of lignin, 3.5% of pectin, 2.0% of uronic acids and 1.5% of ash. (Fig. 2)

Effect of temperature on concentration of hemicelluloses, lignin, phenolics and fufurals by 2% alkali pretreatment method with $Ca(OH)_2$

The effect of different temperatures (30-80^0C) on hemicelluloses, lignin, phenolics and fufurals by alkali pretreatment are shown in Tab.1 Maximum concentration of hemicellulose was observed at 70^0C (32.83±0.13g/100g of dry weight of corncobs) and the concentration of lignin, phenolics and furfurals at 70^0C

was found to be 0.090±0.011g, 0.34±0.01g,and 0.0031±0.00040g per 100g of dry weight of corncobs respectively. Further, it was also observed that above 70^0C, there was an increase in the concentration of all the constituents except hemicellulose. Due to maximum concentration of hemicellulose at 70^0C, further experiments were carried out using 70^0C as optimum temperature.

Table1. Effect of temperature on concentration of hemi celluloses, lignin, phenolics and furfural by 2% alkali pretreatment method with Ca $(OH)_2$

Temp in (^{0}C)	g/100g of dry weight of corncobs			
	Hemi cellulose	Lignin	Phenolic	Furfural
control	19.50±0.30	0.056±0.017	0.14±0.015	0.0016±0.00015
30^0C	19.83±0.45	0.063±0.016	0.23±0.02	0.0022±0.00025
40^0C	20.83±0.44	0.071±0.013	0.25±0.02	0.0025±0.00025
50^0C	28±0.26	0.074±0.008	0.27±0.02	0.0027±0.00030
60^0C	29.16±0.35	0.081±0.011	0.31±0.03	0.0030±0.00030
70^0C	**32.83±0.13**	0.090±0.011	0.34±0.01	0.0031±0.00040
80^0C	31±0.39	0.117±0.015	0.41±0.01	0.0046±0.00020

± indicates Standard deviation of triplicate

Effect of incubation time on concentration of hemicelluloses at 70^0C by 2% alkali pretreatment method with $Ca(OH)_2$

The effect of incubation time on hemicellulose concentration is shown in Table. 2 Incubation period of 5hrs at 70^0C was found to be optimum for maximum yield of hemicellulose (32.81±0.13g/100g of dry weight of corncobs).

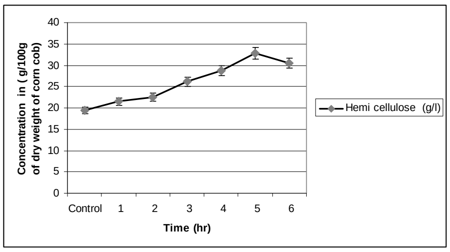

Fig. 3 Effect of incubation time on concentration Hemicellulose at 70^0C by 2% alkali pretreatment method with Ca $(OH)_2$

Table. 2 Effect of temperature on concentration of hemicelluloses, Lignin, Phenolics and Furfural by acid pretreatment method (2% dilute H_2SO_4)

Temp in (^{0}C)	g/100g of dry weight of corncobs			
	Hemi cellulose	Lignin	Phenolics	Furfural
Control	18.33±0.34	2.17±0.12	1.4±0.2	0.020±0.003
70^0C	20.33±0.23	4.9±0.40	2.8±0.3	0.027±0.003
90^0C	22.18±0.21	5.7±0.40	3.6±0.2	0.036±0.002
100^0C	**23.83±0.18**	6.0±0.25	3.8±0.2	0.043±0.003
121^0C	20.82±0.12	7.4±0.20	4.3±0.2	0.052±0.007

± indicates Standard deviation of triplicates

Effect of temperature on concentration of hemicellulose lignin, phenolics and fufurals, by acid pretreatment method (2% H_2SO_4).

The effect of different temperatures (70^0C-121^0C) on hemicellulose, lignin, phenolics and fufurals are shown in Fig.3.The optimum temperature for maximum concentration of hemicellulose was 100^0C. Hemicellulose obtained by acid pretreatment was less (23.83±0.18 g/100g of dry weight of corncobs) when compared to alkali pre treatment (32.83± 0.18 g/100g of dry weight of corncobs). However during acid pretreatment method the concentration of lignin, phenolics, and furfurals was high when compared to alkali pre treatment method. The optimum temperature for maximum hemicellulose yield were found to be at **100^0C**, so further experiments was carried out at this temp.

Effect of incubation time on concentration of hemicelluloses at 100^0C by acid pretreatment method (2% H_2SO_4)

When hemicellulose was treated with 2% dilute H_2SO_4 at 100^0C for different time intervals (0.5h-2.5h), the concentration of hemicellulose was found to be optimum at 1hr (23.81±0.19g/100g of dry weight of corncobs). Further it was observed that, with the increase of incubation time the hemicellulose concentration was decreased. (Fig.4)

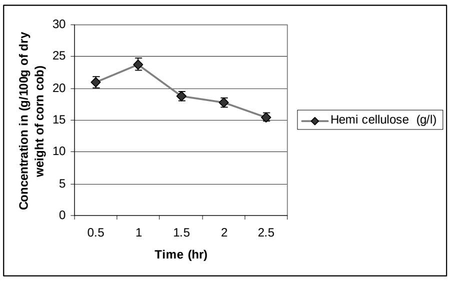

Fig.4 Effect of incubation time on concentration of hemicellulose at 100^0C by acid pretreatment method (2% H_2SO_4)

DISCUSSION

Some of the agricultural residues like corncobs, almond shells, olive stones, rice husks, wheat straw and barley straw were used as feed stocks for the production of xylo-oligosaccharide [12]. Xylan-rich materials are essentially hydrolytic in nature and can be used to produce xylo-oligosaccharides. This can be either carried out by chemical means using acid or base, or catalyzed by enzymes [6]. Chemical hydrolysis is a simple and rapid method for separation of hemicellulosic material, however pretreatment conditions vary with agro-industrial material with respect to the type of chemical agent, concentration, incubation, temperature and time [13,4]. Acid hydrolysis is carried out with different mineral acids such as sulfuric, hydrochloric, nitric, hydrofluoric, acetic and phosphoric acids at high temperature and pressure commonly at 160^oC and 10 atm, respectively

[14]. It can be performed under concentrated (50–70%) or diluted (below 2%) conditions, but generally dilute acid and high temperatures are preferred [15,16]. All sugar liquors obtained by chemical hydrolysis contain furan derivatives such as furfurals and hydroxymethylfurfural (HMF) from the degradation of pentoses and hexoses, respectively[17]. HMF is normally produced in less concentration compared to furfurals during hexoses degradation. This is mainly due to the low quantities of hexose in hemicellulose. Acetic acid, the major aliphatic acid present in chemical hydrolysates, is mostly released from the hemicellulosic acetyl groups. During the acid hydrolysis, a minor part of lignin is also degraded to release a wide range of aromatic compounds including low molecular mass phenolics [18]. Formation of inhibitory compounds became readily apparent for all acid pretreatments, at higher temperatures (160–170°C). Thus, the corn fiber was pre treated with dilute acid (0.5–1.0% H_2SO_4, v/v) at a comparatively lower temperature (121°C) in order to minimize the formation of inhibitory compounds [19]. It is also reported that the sugar degrading products like furfural and HMF in lignocellulosic hydrolyzate can inhibit fermentation of sugars in the hydrolysate [17]. With use of strong alkali solutions, depolymerized xylan maybe extracted from lignocellulosics but the product obtained is completely deacetylated and has very limited solubility in water, hence it is not the preferred hydrolyzing reagent [12]. A large portion of the xylose fraction was degraded during strong alkali pretreatment which was performed at 140°C and 160°C hence strong alkali and high temperatures are not suitable for this purpose[20]. Pretreatment with mild alkali produces reduced quantities of sugar derivatives like furfurals and hydroxyl methylfurfurals[6]. Alkali pretreatment method performed at mild temperatures yields a high mass of hemi cellulose without modifying the cellulose structure substantially, thus allowing improved recovery of glucose during further processing [6]. Mild alkali treatment is an alternative method for the separation of hemicellulose[21,22] and in this method sugarcane bagasse was used as substrate and, they tried to isolate pure cellulose for improved recovery of glucose for further transformation into ethanol. In contrast, present investigation was carried out to isolate pure hemicellulose for its further conversion to xylitol. Moreover, during the process, cellulose and lignin gets separated for further use and it was observed that pretreatment with both concentrated and dilute acid requires high temperature and pressure.

It also produces more amounts of inhibitory compounds, which affect the growth of organisms. To make acid pretreated hydrolyzate suitable for growth of microorganisms it has to go through another process i.e. detoxification which further increases the production cost. With concentrated alkali treatment, hemicellulose yield was very high but removes all the acetyl groups present in xylan which makes hemicellulose insoluble. Most of the available literature suggests acid pretreatment method for separation of hemicellulose but as it has its own limitations alkali pretreatment method gained importance. Though few research papers are available on alkali pretreatment method, their main emphasis was on extraction of cellulose in pure form. Hence, we carried out dilute alkali pretreatment for separation of hemicellulose by using corncobs and achieved satisfactory results.

CONCLUSION

Dilute alkali pretreatment method found to be an effective alternate method for the separation of hemicellulose from corn cobs, as it produces high amount of hemicellulose, less inhibitor compounds and also avoids detoxification step, as the process itself produces less inhibitor compounds, makes the separation economical when compared with acid pre treatment method. In this dilute alkali pre treatment method we could obtain, The hemicellulose concentration of 32.83**±0.13g/100g of dry weight of corncobs (86%)** and the concentration of inhibitors associated with hemicellulose i.e. phenolics, furfurals and lignin 0.34±0.01g/100g, 0.0031±0.00040g/100g and 0.09±0.011 g/100g of dry weight of corncobs, respectively

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